163 Microbiology of the lower respiratory tract of infants with cystic fibrosis using terminal restriction length polymorphism

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The lung disease of Cystic Fibrosis (CF) is characterized by a vicious cycle of infection and inflammation. Both processes appear to be present in the lower respiratory tract (LRT) from an early age in individuals with CF. Previous studies have identified a number of pathogens in LRT specimens obtained from infants with CF but have relied upon the culture of such organisms on general microbiological media. The limitations of culture-based microbiology are now well established and as such, molecular methodologies are required to thoroughly characterize the microbial community present within a given sample. The total microbial community of the CF infant lung is largely unknown at present. A thorough understanding of this community is likely to result in improved protocols for the treatment of CF patients in infancy. Bronchoalveolar lavage fluid (BALF) specimens were obtained from infants (<12 months of age) with CF. Nucleic acids were extracted, bacterial 16S rRNA amplified through PCR and PCR products analysed using Terminal Restriction Length Polymorphism (T-RFLP). T-RFLP bands were identified that corresponded to a range of organisms, including *Pseudomonas aeruginosa*, *Haemophilus influenzae*, Alcaligenes spp. and Streptococcus spp. These findings challenge the current accepted view that *P. aeruginosa* is only present in the CF lung in a minority of infants and and that a limited number of distinct pathogens are present in the LRT.

164 Anaerobic bacteria detected in sputum of cystic fibrosis patients by its cellular fatty acid markers

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Recently it has been shown that the lungs of CF patients are not colonized only by commonly recognized bacteria, such as *Ps.aeruginosa*, but also by a range of potentially pathogenic anaerobic species.

Aim: was to describe the bacterial communities present in sputum of CF children by the gas chromatography and mass spectrometry (GC-MS) method.

Method: Sputum samples were collected from 35 CF patients, 6–17 years old, 17 m. Microbial cellular fatty acids were extracted by chemical procedure and analyzed by GC-MS.

Results: The algorithm of mass spectrometric parameters was developed, which permitted the determination of about 200 known microbial fatty acids, aldehydes and sterols, sufficient for the detection and quantitative determination of more that 150 taxons of clinically significant mucosa microorganisms without precultivation. The participation of 47 microbial taxons, including some fungi and viruses, in the process was confirmed. Anaerobes from a range of species including *Eubacterium (E.moniliforme, E.nodatum, E.sabureum, E.lentum), Propionibacterium freudenreichii, Clostridium ramosum, Actinomyces, Eggertella* were detected in high numbers (3×10^9 cells/ml, 1.4×10^9 cells/ml, 4×10^8 cells/ml, 4×10^8 cells/ml, 1×10^8 cells/ml, 1×10^7 cells/ml, respectively). In comparison: *St.aureus* and *Ps.aeruginosa* – 4.8×10^7 cells/ml). The concentration of other anaerobes (*Bacteroides, Porphyromonas, Prevotella*), mentioned as potentially significant in previous works, was not clinically important in our patients.

Conclusion: Such anaerobes as *Eubacterium*, *Propionibacterium freudenreichii*, *Clostridium*, *Actinomyces* and *Eggertella* could play a clinical role to CF patients.

165 Evaluation of the effectiveness of the modified *Burkholderia cepacia* selective agar (BCSA) for isolation of *B. cepacia* complex from respiratory secretion in CF patients

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Burkholderia cepacia complex (Bcc) has been suggested as important pathogens in lung disease of Cystic Fibrosis (CF) patients, and selective medium is necessary for their recovery specially in patients harboring mucoid *P. aeruginosa.* We evaluate the effectiveness of a home made selective medium modified from BCSA original formulation, as an option for locations that have limited resources. From January 2005 to January 2008 we analyzed 1368 sputum and oropharyngeal secretion samples, from 216 CF patients attending the outpatient Pediatric CF reference center from the State University Hospital, Unicamp, Brazil. The BCSA agar original formulation was modified using only 2.5 mg/L of vancomycin and 600.000U of injectable Colistin as selective antibiotics and keeping the other reagents. *P. aeruginosa* ATCC 27853, *B. cepacia* ATCC 25416 were used for quality control. From January 2005 to October 2006 we detected 8/152 (5.2%) patients with Bcc without selective medium, 6 cases chronic and 2 with a single sample. In next period from November 2006 to September 2007 with the modified selective BCSA medium, 7 new cases were detected and the previous 5 Bcc chronic infection patients (one of the 6 chronic patients died), 12/153 (7.84%). In the 3rd period from September 2007 to January 2008 we compared the standard BCSA OXOID® and the modified BCSA for 112 patients and only one new patient was detected in both media. These results confirm the effectiveness of the modified selective medium, easy preparation, low cost and the usefulness in microbiology routine for CF patients.

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166 Unusual species distribution of *Burkholderia* isolates from Danish cystic fibrosis patients

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Preserved isolates from 1994 to 2007 of *Burkholderia* species from 47 Danish patients from two Centers were investigated (one isolate per patient). Specific identification of 46 isolates was perfomed by partial sequencing of two housekeeping genes. Comparison of either atpD or recA sequences with reference strains gave identical results. Forty isolates were identified as *B. multivorans*, two isolates as *B. cenocepacia*, two as [*B. cenocepacia*] genomovar IIIB, and one as *B. anthina*. The last isolate was identified as *B. gladioli* by 16S rDNA sequence. Genotyping by PFGE revealed little cross-infection among Danish CF patients.

Only 4 isolates (9%) were classified as *B. cenocepacia*/genomovar III, whereas 85% of the isolates were classified as *B. multivorans*. This species distribution is in contrast with reports from other CF centers in Europe and North America, where clones of *B. cenocepacia* and occasionally *B. cepacia* have spread among patients. A preponderance of *B. multivorans* among patients with CF may represent the sporadic acquisition of *Burkholderia* strains from the environment, at least in Denmark.

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